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13. ABSTRACT ***(Maximum 200 Words)***

The intracellular polyamines, spermidine, spermine, and putrescine, play an important role in the proliferation and death of normal and malignant cells. As a consequence work has focused on development of inhibitors of this metabolic pathway, particularly diethylnorspermine (DENSpm). In the first year a phase II trial of DENSpm for women with advanced breast cancer has enrolled nine women. Response analysis is in progress and no excessive toxicity has been observed. Collection of DENSpm- and control-treated breast cancer tissues derived from discarded mastectomy tissues has begun and 23 pairs that are potentially suitable for analysis have been stored; collection continues. Proposed testing of DENSpm in animal models has been delayed because of our move to a new building with new animal facilities in the middle of the first year and a current concern about whether the cell line proposed is contaminated in some way. It is expected that they will begin shortly. Proposed chemoprevention studies are expected to begin in the second year as initially stated.

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INTRODUCTION

The intracellular polyamines, spermidine, spermine, and putrescine, play an important role in the proliferation and death of normal and malignant cells. As a consequence work has focused on development of inhibitors of this metabolic pathway. The purpose of the ongoing studies is to evaluate the therapeutic and preventive actions of one polyamine analog, DENSpm (N^1,N^{11}-diethylnorspermine) in breast cancer. This will be accomplished through four related technical objectives. They include: 1) to test the therapeutic efficacy of DENSpm against human breast cancer cell lines in nude mice, 2) to use transient organ cultures of normal and malignant human breast tissues to assess effects of DENSpm on biological and pharmacological parameters relevant to antineoplastic activity, 3) to evaluate the efficacy of DENSpm in a phase II trial in women with metastatic breast cancer, and 4) to evaluate the chemopreventive activity of DENSpm in the rat mammary tumor model.

BODY

Technical Objective 1: To test therapeutic efficacy of DENSpm against estrogen receptor-positive MCF-7 and estrogen receptor-negative MDA-MB-468 human breast cancer cell lines growing in nude mice and assess tumors for possible biological or pharmacological end-points which predict subsequent tumor response.

After grant funding in September, 2000, implementation of the proposed animal studies was purposely delayed as our Cancer Center was to move into a new building with new animal facilities in December, 2000 and we did not wish to have to move animals into a new facility mid-experiment. The building move was delayed until January, 2000. Thus, in the spring of 2000 (after the animal facility had operated successfully for several months) studies began. Five female nude mice were inoculated with MDA-MB-468 cells to serve as donor animals for tumor fragments for trochar implantation. Tumors were grown to 1 cm^3. Unfortunately during that time, other experiments in the lab raised uncertainty about whether these cells have been contaminated or changed biological behavior as RT-PCR assays suggested that they were estrogen receptor-positive rather than estrogen receptor-negative. Because of this uncertainty, the animals were sacrificed and studies are in progress to completely characterize the cells before embarking on new animal studies. The plans for this technical objective are unchanged and animals studies should begin again shortly.

Technical Objective 2: To use transient organ culture of normal and malignant human breast tissues to assess the effects of DENSpm on biological and pharmacological parameters relevant to antineoplastic activity as identified in Technical Objective 1.

The intent was to incubate normal tissue and malignant tumor tissues with or without 10 uM DENSpm in a transient organ culture for 24 hours. Tissues would then be harvested and fixed for subsequent immunohistochemical studies. To date 23 pairs of DENSpm- and control- treated malignant tissues have been obtained, 18 infiltrating ductal cancers, 3 infiltrating lobular tissues, and 2 ductal carcinoma in situ lesions. In some cases the tumor tissue obtained was so small that it was not possible to have a concurrent untreated control and sections from the paraffin block of diagnostic material must be used as a control. A single assay (immunohistochemistry for SSAT) has been undertaken in a subset of the tumors. It has shown evidence of inducibility of SSAT by DENSpm in some cases. However, unlike the situation with lung cancer, background expression of SSAT in untreated tissues is also noted. It is not clear whether this is a real phenomenon or whether is represents a characteristic of the assay when applied to fixed tissues.

For these reasons our plan is to continue to accrue samples in order to maximize the number of true matched control and treated tissues. In addition preclinical studies will be undertaken using human breast cancer cell lines in culture that are DENSpm-treated, harvested, pelleted, and formalin fixed to mimic the treatment of the human tissues. These materials will be used to refine the immunohistochemical assay.

Technical Objective 3: To evaluate the efficacy of DENSpm in a phase II trial in women with metastatic breast cancer.

The protocol J9951 (An Open-Label, Single Center, Phase 2 Study of Intravenous Diethylnorspermine - DENSPM in the Treatment of Patients With Previously Treated Metastatic Breast Cancer) was originally approved by the Johns Hopkins University IRB in mid-1999. However, we did not receive final approval by the US AMRMC to proceed with study activation until early 2000. The site initiation visit by Parke-Davis took place on February 24, 2000, and the first patient was enrolled on April 18, 2000. Since then a total of nine patients have been enrolled in this study. All patients had evidence of progressive metastatic breast cancer, and had been treated with at least one but no more than two prior palliative chemotherapy regimens. These nine patients have thus far received a total of 24 cycles of therapy (median 2, range 1-4). Each cycle of therapy consists of a 15-minute infusion of DENSpm 100 mg/m^2 daily x 5. As of September 11, 2000, the first six patients have had disease progression, while the last three patients enrolled remain on study (cycles 1, 3, and 4, respectively). Patient 9 started treatment on September 11, 2000. Thus far, there have been two hospitalization events, both in patient 5, one episode of uncomplicated catheter site infection (cycle 1) and one episode of grade 3 gastrointestinal toxicity (cycle 2). Both resolved following a brief hospital admission. This last event was similar in nature to what had been observed at the phase I trial at an MTD of 145 mg/m^2. The primary objective of this study is to estimate the proportion of patients who are progression free at 4 months. According to the two-stage statistical design of this trial, no formal response assessment will occur until 17 patients (15 eligible) are enrolled. If two or more patients are progression free at 4 months on study among these 15 eligible patients, an additional 17 patients will be enrolled. Ultimately, this compound will be considered worthy of further study if six or more patients (about 20%) are progression free after 4 months on study. Finally on August 8, 2000, we were notified by Parke-Davis (now Pfizer) that the IND of DENSpm is being transferred to GelTex. Parke-Davis (Pfizer) is responsible for monitoring the first eight patients enrolled; subsequent participants will be monitored by GelTex.

Technical Objective 4: To evaluate the chemopreventive activity of DENSpm in the DMBA rat mammary tumor model.

Implementation of this technical objective was not planned until year 2 as results from Technical Objective 1 are needed to guide the design of these studies. It is expected that they will begin as described later in year 2.

KEY RESEARCH ACCOMPLISHMENTS

- Activation of a phase II clinical trial to assess the efficacy of DENSpm in women with advanced breast cancer.

- Successful implementation of a plan to collect DENSpm-treated and untreated transient organ cultures harvested from tumor tissues from discard mastectomy specimens.

REPORTABLE OUTCOMES

None to date

CONCLUSIONS

A series of laboratory and clinical studies to evaluate the effects of a polyamine analog, diethylnorspermine (DENSpm), on growth of human breast cancer cells in nude mouse models, transient organ cultures derived from human breast cancer tissues, and women with advanced breast cancer has begun. When complete these studies should shed light on the possibility that this class of compounds may have promise as agents for the treatment and prevention of breast cancer.

REFERENCES

None

APPENDICES

None